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Allometric Equations for Estimation of Ash-free Dry Mass from Length Measurements for Selected European Earthworm Species (Lumbricidae) in the Western Great Lakes Region

Abstract.—In the last decade the invasion of European earthworms into previously worm-free glaciated areas of North America has stimulated research into their impacts on native forest ecosystems in the region. As a first approximation, the impacts of invading earthworms are related to their biomass. However, direct measurements of biomass can be significantly affected by the moisture conditions under which the earthworms are collected and their relative gut contents. Ash-free dry mass is the best standardized measure of earthworm biomass, but requires the destruction of specimens. This paper presents five allometric equations that allow for estimation of ash-free dry biomass based on length (mm) measurements for European earthworm species (Lumbricidae) commonly seen in the United States.

Introduction

In the last decade the invasion of exotic earthworms into previously worm-free glaciated areas of North America has stimulated research into their impacts on native ecosystems in the region (Hendrix and Bohlen, 2002). European earthworms have been invading the North American continent since European settlement (Gates, 1982). In the previously worm-free glaciated regions of North America their appearance in native forest ecosystems is often associated with a cascade of changes in ecosystem function and structure (Hendrix, 1995; Burtelow *et al.*, 1998; Gundale, 2002; Bohlen *et al.*, in press). Alban and Berry (1994) documented rapid changes in soil morphology, carbon and nitrogen in response to earthworm invasion of a birch-aspen forest. Scheu and Parkinson (1994) demonstrated how earthworm invasion can affect plant growth in an aspen forest. In lodgepole pine forests, McLean and Parkinson (1997) documented decreases in organic matter content, total nitrogen, carbon, basal respiration and metabolic quotient (qCO_2) with increasing earthworm biomass. To a first approximation, these changes are proportional to earthworm biomass. Research investigating the effects of the invasion by European earthworms on a wide variety of ecological parameters and ecosystems necessitates an efficient determination of earthworm biomass. Preservation of collected specimens for future reference is often desirable. Therefore, an alternative to direct measurement is needed which does not result in the destruction of the specimens. Individual earthworms within a species vary in size and body proportions, making the total number of individuals a poor measure of total biomass of a species (McLean and Parkinson, 1997).

Earthworm fresh mass can vary greatly depending on the moisture status of the environment in which they are found (Lee, 1985). Earthworms preserved in formalin commonly lose a significant proportion of their fresh body mass (Lee, 1985). Variability in gut content can account for up to 20% of both fresh and dry mass measures of biomass (Lee, 1985; Edwards and Bohlen, 1996). For these reasons, ash-free dry mass measures of biomass which remove gut contents from the dry mass measurement are the most accurate and provide data that is comparable between different sites and under different conditions (Edwards, 1998). Although models relating length to biomass for some invertebrate taxa are available in the literature (Rogers *et al.*, 1977; Schoener, 1980), including a few equations relating length to either fresh or dry mass for selected genera or species of terrestrial Oligochaeta (Lumbricidae) (Lee, 1985; Collins, 1991), equations relating length to ash-free dry mass measures of biomass do not exist. This paper presents five allometric equations that allow for estimation of ash-free dry biomass based on length (mm) measurements for European earthworm species (Lumbricidae) commonly seen in the United States.

Earthworm assemblages in a given site can range widely in species composition. Different earthworms within a species vary in both size and proportion. Therefore, specific equations relating length to ash-free dry mass are needed for each species or group of closely related species (Bohlen, pers. comm.). The allometric equations presented here are for some common earthworm species found in the western Great Lakes region and beyond (Reynolds, 1995; Reynolds *et al.*, 2002) including *Aporrectodea* species, *Octolasion tyrtaeum*, *Dendrobaena octaedra* and *Lumbricus* species.

TABLE 1.—Earthworms used in determining allometric equations

Species	# adult	# juvenile	Total #	Length range (mm) adult/juvenile
Octolasion tyrtaeum	20	37	57	15–44/13–39
Aporrectodea species[a]	72	169	241	30–86/10–77
Lumbricus species[b]	23	65	88	30–137/15–125
Dendrobaena octaedra	14	143	157	18–23/10–22

[a] Included *Aporrectodea rosea*, *A. caliginosa* and *A. tuberculata* adults and juvenile *A.* where species designation was impossible

[b] Included *Lumbricus rubellus* and *L. terrestris* adults and juvenile *Lumbricus* where species designation was impossible

METHODS

Earthworms were collected in four sites on the Chippewa National Forest in north central Minnesota as part of a study investigating the impacts of the invasion by European earthworms on hardwood understory plant communities. The four study sites are similar with respect to overstory composition, soils and stand history. Sugar maple (*Acer saccharum*) is the dominant tree species with yellow birch (*Betula alleghaniensis*), paper birch (*B. papyrifera*) and basswood (*Tilia Americana*) as secondary species. Soils are deep, well-drained and light colored Eutroboralfs (Warba series) associated with the Guthrie Till Plain (USDA, 1997).

Earthworms used to determine the allometric equations were collected in the fall of 2000 by liquid extraction using a mustard solution (Lawrence and Bowers, 2002; Linden and Wallach, unpubl. data). Specimens were preserved in the field with 70% isopropyl alcohol and transferred to 10% formalin at the end of each day. Well-preserved and undamaged specimens were selected for determination of the allometric equations for each species with a range of lengths and sexual maturities (Table 1).

Each earthworm collected was measured to the nearest 1.0 mm. Length was measured on the longest axis of the straightened individual. Individuals were dried for 24–48 h at 60 C and weighed to the nearest 0.0001 g. Individuals were then ashed at 500 C for a minimum of 4 h. The mass of the remaining ash was measured and subtracted from the dry mass measurement to get the ash-free dry mass of each individual. For small specimens (<40 mm) where the dry mass was <0.009 g, the mean ash-free dry mass was determined from 4–10 specimens of equal length combined into a single sample. Where multiple specimens of the same length were used to determine a mean ash-free dry mass, only one resulting data point was used in the regression analysis.

Regression analysis of natural log-transformed data was used to determine the equations describing the relationship between length and ash-free dry mass for each species or species group (Gould, 1965; Burton, 1998). While a high level of predictive power can often be achieved using non-transformed data ($r^2 \geq 0.90$), the relation of a volumetric measure such as biomass to a linear measure such as length necessitates the use of a power function (Gould, 1965). Earthworm ash-free dry mass (grams) and length (mm) were fit to the natural logarithmic transformation of the standard allometric growth function yielding:

$$\ln(ash-free\ dry\ mass) = b\ \ln(length) + \ln a \tag{1}$$

Eq. (1) not only increases the predictive power (r^2), but also improves the fit of the model at both the small and large extremes of earthworm length. There were no significant differences in either the slope or intercept of the regressions of adults versus juveniles for any species (Glantz, 1992) so a single regression was calculated for each species, including both juvenile and adult specimens (Fig. 1).

RESULTS AND DISCUSSION

Octolasion tyrtaeum was the only species whose allometric equation of mass vs. length was significantly different from the other species (Fig. 1). This is not surprising since *O. tyrtaeum* generally appears to have

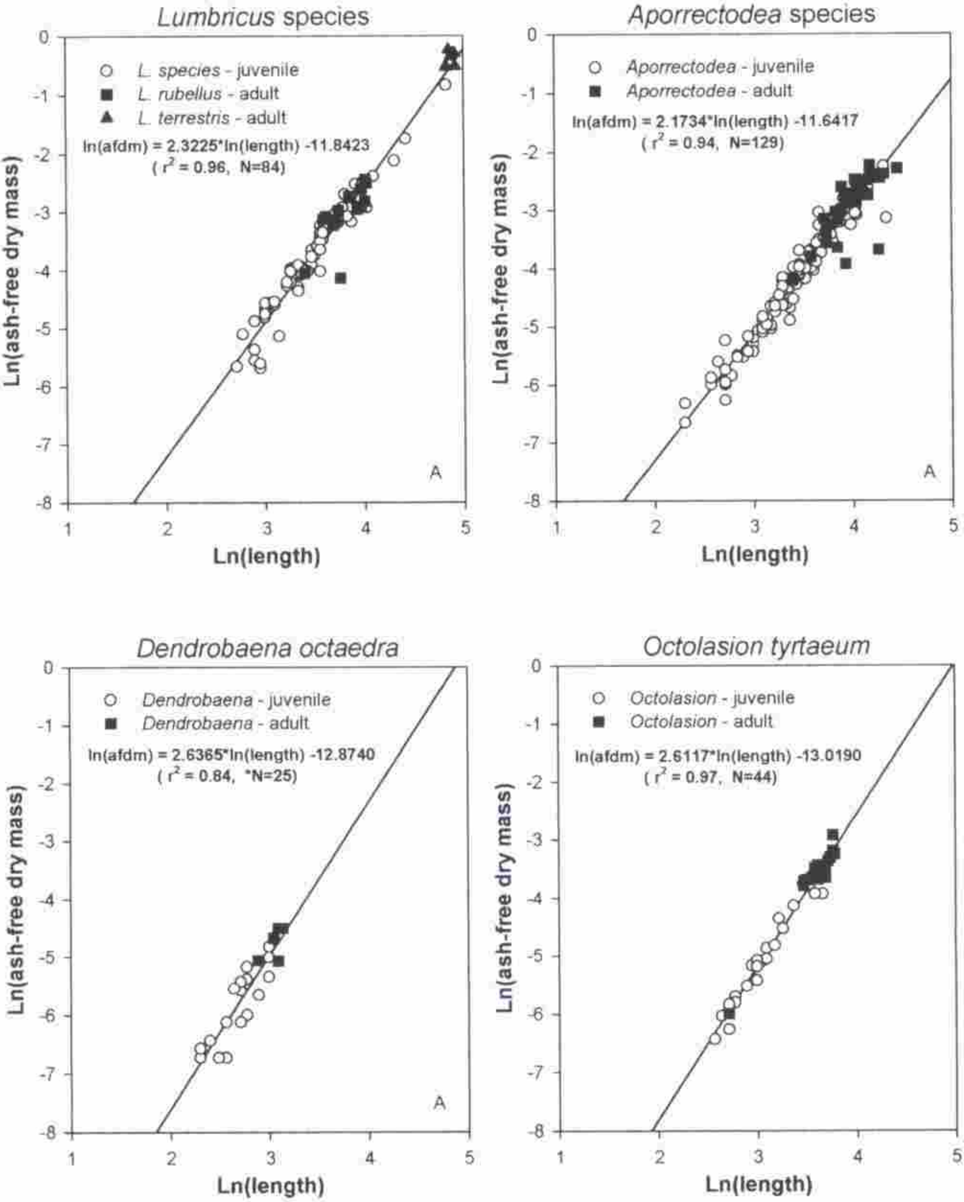

FIG. 1.—Individual allometric regressions relating measured earthworm length (mm) to ash-free dry mass (grams) for four common European earthworm species or genera. Species groups with the same letter (A) do not have significantly different regressions ($P < 0.01$). * The N value for *Dendrobaena* represents the number of data points rather than the actual number of worms. This was due to the need to determine mean ash-free dry masses for small specimens by combining multiple individuals

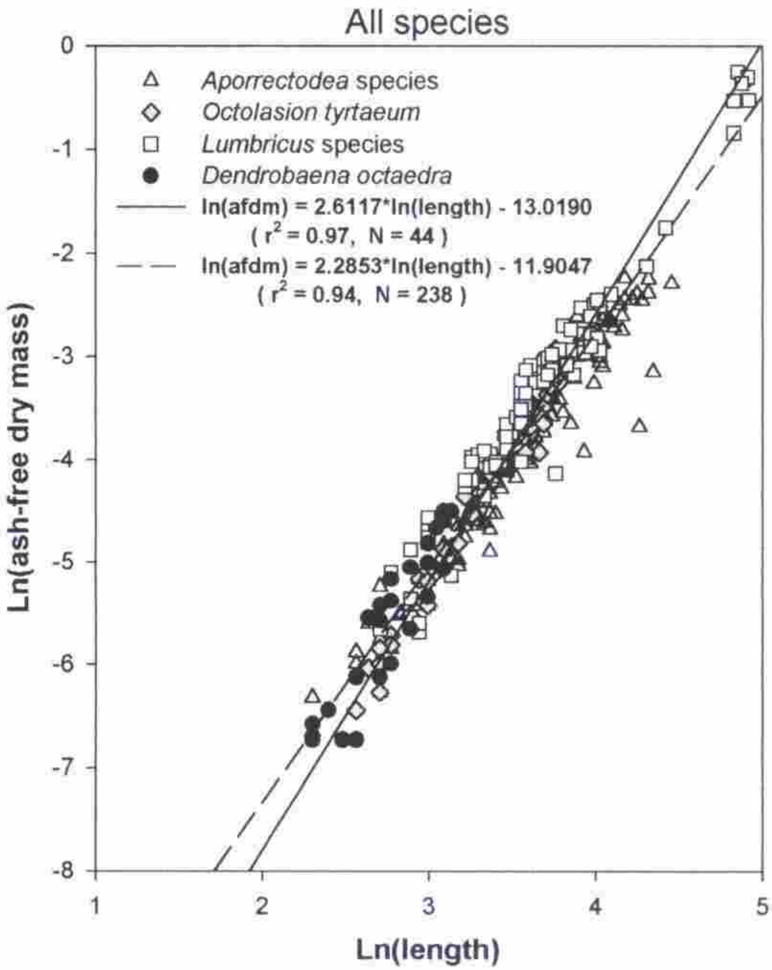

FIG. 2.—Generalized allometric regression relating measured earthworm length (mm) to ash-free dry biomass (grams) for European earthworms. The dashed line represents the regression for *Aporrectodea* species, *Lumbricus* species and *Dendrobaena octaedra* combined. The solid line represents the regression for *Octolasion tyrtaeum* which was significantly different ($P < 0.01$) than all other species and the generalized regression

a more uniform diameter from head to tail, so biomass would be expected to increase more rapidly with length than in the other species which have a more tapered body shape.

The allometric equations for *Aporrectodea* species, *Lumbricus* species and *Dendrobaena octaedra* individually were not significantly different from each other, indicating that individual equations relating length to ash-free dry mass may not be necessary for these species (Figs. 1, 2). The size range of *Aporrectodea*

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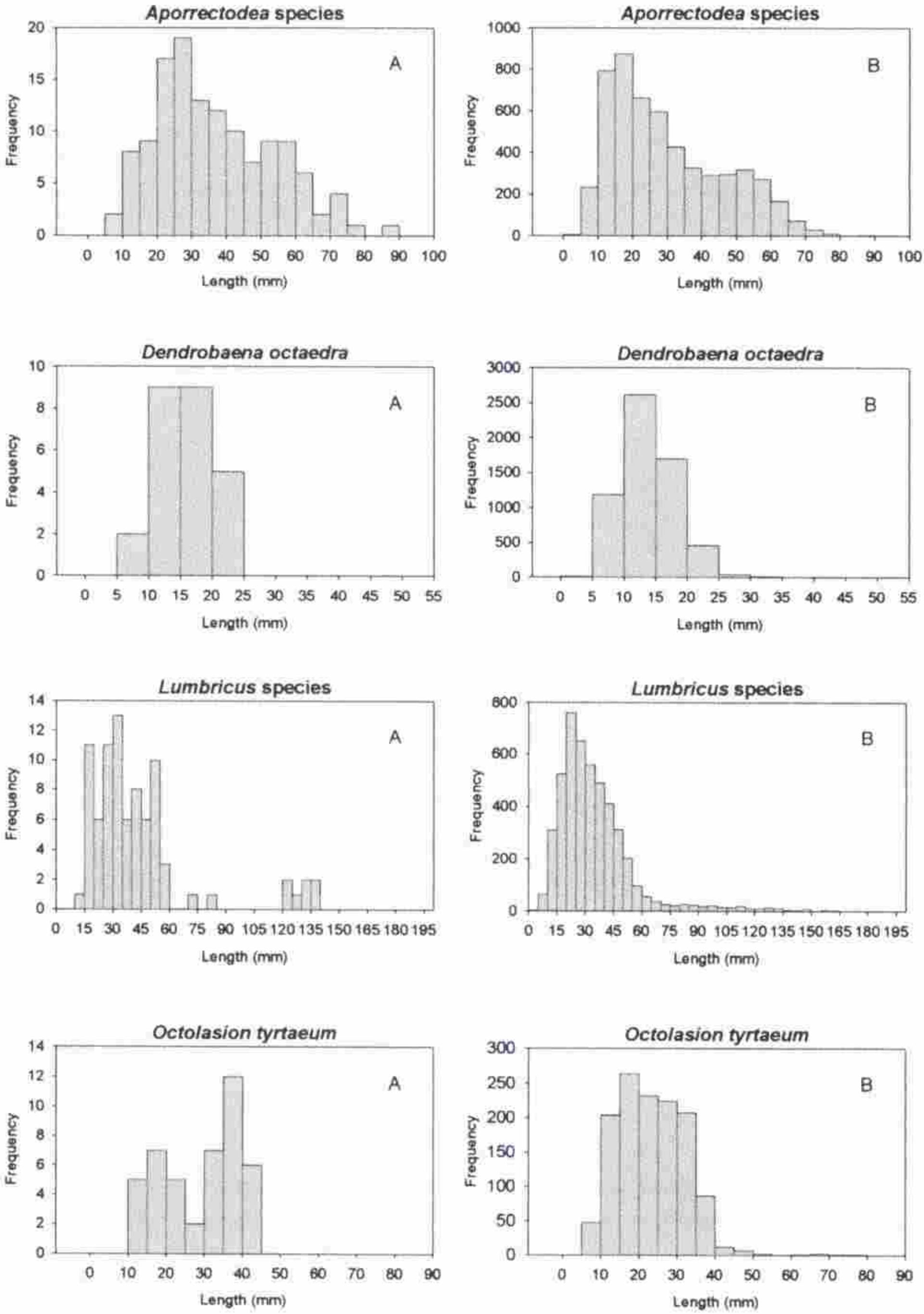
FIG. 3.—Graphs labeled (A) are the frequency distributions of earthworms used to develop the allometric equations for each species group. Graphs labeled (B) are the frequency distributions of all earthworms collected in 1998 through 2001 for each species group


Aporrectodea species
A
B
Dendrobaena octaedra
A
B
Lumbricus species
A
B
Octolasion tyrtaeum
A
B
Frequency
Length (mm)

and *Lumbricus* specimens used to determine the allometric equations overlapped completely, while the size range of *D. octaedra* specimens was smaller than those of *Aporrectodea* and *Lumbricus*. The lower r^2 for the regression for *D. octaedra* alone is likely due to the small size of that species and the fact that even when combining 10 individuals, the dry masses and ash masses were in the range of 0.001 to 0.01 grams making accurate weighing difficult. Inclusion of all three genera (*Aporrectodea*, *Lumbricus* and *Dendrobaena*) in the combined regression yields a generalized allometric equation with high predictive power over a wide range of earthworm sizes.

The maximum lengths of specimens used to determine the allometric equations here are smaller than those reported in taxonomic literature for each species (Reynolds, 1977). However, the range in length used here represented the full range of specimens collected at our study sites over a 4 y period (Fig. 3). This speaks to the range in variability that is possible within species in different contexts. The regression equations shown here may not provide accurate estimates of biomass for individual specimens larger than those used to develop the regressions. While it is not yet known how different soil types may affect earthworm size and these allometric relationships, ash-free dry weight measures would be expected to be the least variable across different soil types because the gut contents are removed from the measurement.

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CINDY M. HALE,[1] PETER B. REICH AND LEE E. FRELICH, University of Minnesota, Department of Forest Resources, 1530 Cleveland Ave. N., 115 Green Hall, St. Paul 55108-6112. *Submitted 28 March 2003; accepted 19 August 2003.*

[1] Corresponding author, present address: The Natural Resources Research Institute, 5013 Miller Trunk Highway, Duluth, Minnesota 55811-1442